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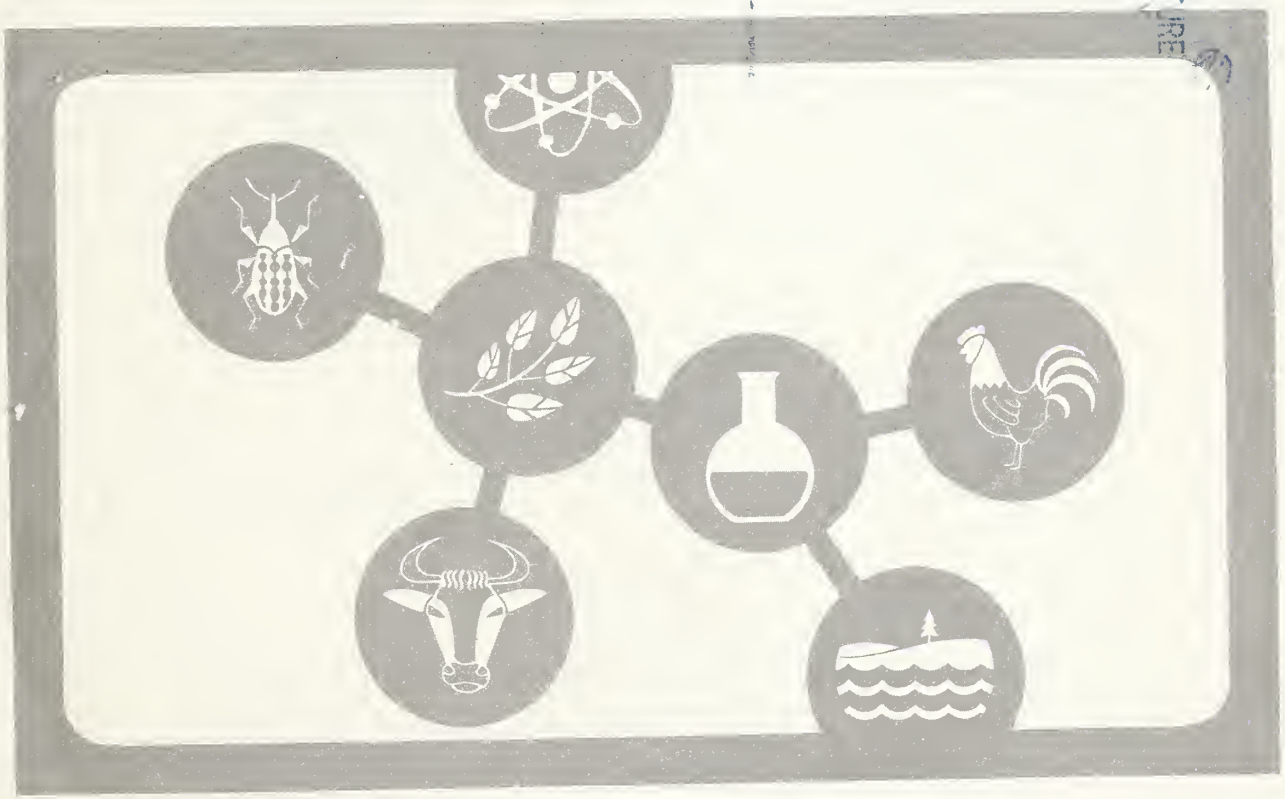
A Study of Nine Corn-Based Food Blends Formulated by Computer for Maximum Protein Quality



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CONTENTS

ILLUSTRATION

TABLES

A Study of Nine Corn-Based Food Blends Formulated by Computer for Maximum Protein Quality

By R. E. Hayes,[1] J. J. Spadaro,[2] J. I. Wadsworth,[3] and D. W. Freeman[4]

ABSTRACT

Nine food blends formulated by computer for maximum protein quality by chemical score and two formulated according to U.S. Food-for-Peace Program (Public Law 480) guidelines were assessed for protein quality, microbial content, texture, color, and flavor (except for one blend) and for storage effects on most of these factors. Protein efficiency ratio (PER) was more sensitive than net protein ratio (NPR) in reflecting amino acid profile differences. Significant correlation of unadjusted PER with chemical score was found over the broad chemical score range obtained by including data from other foods with those representing the 11 corn-based blends but not over the rather narrow chemical score range represented by the blends alone. During storage: little protein degradation of the blends occurred, according to available lysine values; blends without dairy product had the best color and flavor stability; microbial levels mostly decreased but sometimes underwent low magnitude increases that were unimportant in terms of spoilage or public health; at 25° C, high free fatty acid values were found in cottonseed blends without notable effect on flavor; at 25° C, high peroxide values were associated with peanut blends, with probable adverse flavor effect. Corn–cottonseed(with cottonseed oil)–lysine-monohydrochloride had the highest PER of the 11 blends, and flavor quality was comparable to or better than the Public Law 480 blend corn-soy-milk for all storage conditions tested. Index terms: available lysine, corn-soy, corn-soy-milk, food blend formulation, food color stability, food deterioration, food flavor stability, food protein quality, food storage, free fatty acid values, microbial levels in food, net protein ratio, NPR, PER, peroxide values, protein efficiency ratio, protein foods, Public Law 480.

INTRODUCTION

Public Law 480 (known also as the U.S. Food-for-Peace Program) was enacted in 1954 as a mechanism for distribution, on concessional sales terms, of American agricultural commodities to food-needy world areas. This law has undergone several changes in philosophy and mode of operation since its inception. One major change occurred in August 1966 with the introduction of formulated foods into the program (Senti 1969). Before then, nonfat dry milk was the only food permitted that was particularly suitable as a dietary supplement for meeting the protein needs of weanling infants and preschool children (Senti 1972). Problems with variability in supply and acceptance of nonfat dry milk led to the inclusion of formulated foods under Public Law 480 as alternate protein supplements suited for child feeding in develop-

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ing countries. Guidelines were established that detailed the nutrient composition of the new foods. To meet these specifications, it was necessary to add vitamins and minerals to the basic food commodities of the mixture, which included a cereal, an oilseed, vegetable oil, and in some instances a dairy protein component, such as nonfat skim milk. Corn-soy-milk, corn-soy, and wheat-soy are food blends that have been used in significant quantities under the U.S. Food-for-Peace Program.

To investigate a potential human food use for peanut and cottonseed meals, from which oil was removed by pressing or solvent extraction, personnel at the U.S. Agricultural Research Service's Southern Regional Research Center in New Orleans began research on food blends of the Public Law 480 type. Either peanut flour or cottonseed flour, prepared from the meal, can potentially replace soy flour as a high protein contributor to such blends. Neither peanut nor cottonseed meal is used to an appreciable extent in human food at present in the United States. But, with an expanding population in developing countries, it will likely be necessary to use such potential human food resources more efficiently.

The first stage of this research in the development of peanut- and cottonseed-containing food blends was to establish a rapid computer formulation procedure to supplant the lengthy trial-and-error approach for determining the ingredient proportions needed to achieve best protein quality in the blends. The best protein quality blends, as predicted by the computer, could then be screened in experimental studies to confirm nutritional suitability by animal assay of protein quality and also to assess organoleptic quality and storage stability.

In human (and animal) nutrition, not only the quantity of protein but also its quality is important in defining requirements for this nutrient. Protein quality may be defined as the efficiency with which a protein (or amino acid mixture derived from various protein components) is used by the animal body for growth and maintenance (Joint FAO/WHO *AD HOC* Expert Committee 1973). Protein quality is largely determined by its amino acid composition. The parameter used to quantify protein quality in the computer formulation was chemical score. The chemical score of a blend is defined as the amino acid score of the most limiting essential amino acid (the lowest amino acid score of the essential amino acids). For each essential amino in a blend, the amino acid score is milligrams of amino acid per grams of protein in the blend multiplied by 100 and then divided by the milligrams of amino acid in the FAO/WHO reference pattern. The FAO/WHO Expert Committee on Energy and Protein Requirements has established that the chemical score may be taken as a first approximation to the probable utilization efficiency of a protein or protein mixture by children.

Hayes et al. (1978), in the first paper of this research series, described the use of a flexible simplex search optimization scheme for adjusting blend ingredient proportions to meet certain compositional and ingredient level requirements and also to achieve the best possible protein quality, as measured by chemical score. But the flexible simplex pattern search had certain inherent inefficiencies that made it more impractical with increasing number of blend ingredients. Wadsworth et al. (1979), in the second paper of this series, described a more efficient computer optimization approach, to achieve the same formulation results, using linear programing techniques. The principal purpose of applying these computer optimization procedures was to use them as a rapid means of prescribing potential Public Law 480 blends of likely high protein quality. But it is apparent that this approach is also generally applicable to food and feed mixtures.

The present phase of this research involves experimental evaluation of corn-based blended foods, of the Public Law 480 type, whose ingredient proportions were predicted by computer in the manner described. To limit experimental samples to a manageable number, corn was the only cereal we used. In addition to cornmeal, all blends contained one or more oilseed flours, added vegetable oil, and fortifying mixtures of vitamins and minerals. The oilseed flours used were peanut (types from two processes), soy, glandless cottonseed, and mixtures of soy and glandless cottonseed. Some of the blends contained a dairy product, either nonfat dry milk or whey protein concentrate. Some contained lysine monohydrochloride.

Two of the blends, corn-soy-milk and corn-soy, represented Public Law 480 formulations. The other nine blends were computer-formulated to achieve maximum protein quality by chemical score.

The blends were evaluated for protein quality

by chemical and animal assay; for particle size and consistency as a gruel; and for storage behavior (with and without added antioxidants) of color, flavor (except for one blend), microbial levels, and chemical stability.

METHODS AND MATERIALS

Blend Preparation

Blend components were selected on the basis of the initial study in this series (Hayes et al. 1978). Abbreviated designations for the 11 blends are as follows:

Abbreviation	*Blend mixture*
CSM	Corn-soy-milk.
CP(pre)WL	Corn-peanut(prepressed solvent extracted)-whey-lysine-monohydrochloride.
CP(full)	Corn-peanut(full solvent extracted)-whey-lysine-monohydrochloride.
CCotW	Corn-cottonseed-whey.
CCot(3)SW	Corn-cottonseed(3×soy)-soy-whey.
CCotSW	Corn-cottonseed(=soy)-soy-whey.
CCot(⅓)SW	Corn-cottonseed(⅓ soy)-soy-whey.
CSW	Corn-soy-whey.
CS	Corn-soy.
CCot(soy)L	Corn-cottonseed(with soy oil)-soy-lysine-monohydrochloride.
CCot(cot)L	Corn-cottonseed (with cottonseed oil)-soy-lysine-monohydrochloride.

Generally, we used standard methods for analyses. But we did modify the PAG (Protein Advisory Group) guideline's sampling plan for the microbiological tests. This plan was established for ascertaining microbial levels in factory shipments and involves taking multiple samples. In the present study, however, we used duplicate assays of a single sample. Using the data from the proximate analyses (table 1) and amino acid analyses, we computer-optimized the blend compositions (except for CSM and CS) to obtain the best chemical score consistent with the nutrient and commodity levels following corn-based-formulation goals:

Table 1.—Proximate and sieve analyses[1] of blend ingredients containing amino acids

Ingredient	Proximate analyses						Sieve analyses, percentage passing through U.S. sieve No.—		
	H_2O[1] (%)	Lipids (%)	Protein (N× factor (%)	Ash (%)	Crude fiber (%)	CHO by difference (%)	60	80	100
Cornmeal, processed, gelatinized	9.7	0.4	8.6	0.3	0.7	80.3	....	....	17.4
Cottonseed flour, glandless, defatted.	4.9	1.5	51.1	6.7	2.6	33.2	....	....	95.8
Peanut flour, prepressed solvent extracted.	6.6	.3	52.8	4.9	3.9	31.5	....	....	50.0
Peanut flour, full solvent extracted.	2.5	1.8	53.6	5.1	4.3	32.7	....	....	40.0
Soy flour, defatted, toasted	6.5	1.2	48.3	5.9	3.5	34.6	....	....	97.3
Milk, nonfat, dry	5.0	.0	36.3	7.8	.1	50.8	98.4	92.2	87.9
Whey protein concentrate	6.5	.0	31.8	7.2	.1	54.4	96.7	91.9	89.9
L-Lysine monohydrochloride	.0	.0	69.6	.0	.0	30.4	....	....	....

[1]For proximate analyses, AOCS (American Oil Chemists' Society 1979) procedures were used for H_2O, lipids, crude fiber, and nitrogen (protein). The AOAC (Association of Official Analytical Chemists 1975) procedure was used for ash. The standard ASTM (American Society for Testing and Materials Committee E-29 1972) procedure was followed for sieve analyses. Samples were split with a Jones-type sample splitter. A mechanical sieve shaker with tapper (Ro-Tap testing sieve shaker, W. S. Tyler Co.) was used in the analyses with U.S. standard sieves. Optimum sample weight was found to be 50 grams and optimum shaking time, 15 minutes. The choice of No. 100 screen for characterizing cereal and oilseed ingredients was based on the work of Bookwalter et al. (1977). The cornmeal ingredient, however, was considerably coarser than the flours described in that paper. The choice of Nos. 60, 80, and 100 screens for dairy ingredients was based on recommendations by personnel from Stauffer Chemical Co., Westport, Conn., and Mid America Farms, Springfield, Mo.

Factor		Nutrient or commodity level
Protein	%	20.0
Lipids	%	≥6.6
Whey protein concentrate (in blends containing this ingredient)	%	≤15.0
Mineral premix	%	2.7
Vitamin premix	%	0.1
Protein quality	%	Equal to or better than comparable blends now used in the Public Law 480 program.

The levels outlined above, except for the whey protein concentrate level, met both the general U.S. Department of Agriculture guidelines for gruel-type foods (Senti 1969, 1972) and the present commodity specifications for corn-based blends (U.S. Agricultural Stabilization and Conservation Service 1978a, 1979). Whey protein concentrate has been permitted at a lower value of 5%, as contrasted to the listed value of ≤15%, in instant CSM and sweetened, instant CSM (U.S. Agricultural Stabilization and Conservation Service 1978a, 1979) were used for ingredient proportions. Each blend combination was formulated with and without antioxidants—BHA butylated hydroxyanisole) at 0.0022% plus BHT (butylated hydroxytholuene) at 0.0022%—as required by the corn-soy-milk specification (U.S. Agricultural Stabilization and Conservation Service 1979).

Separate batch mixtures were made for each of the blends. For each batch, components were mixed for about 1 hour at 133 revolutions per minute on a mechanical drum roller in a stainless steel drum fitted with a stainless steel baffle. Before drum rolling, the oil and vitamin premix were blended into cornmeal with a Hobart mixer. Each batch of a given blend was packaged into various-sized glass jars having aluminum-lined screwcaps for minimizing corrosion to make available quantities required for specific analyses. The jars were divided into lots for initial tests, for use in various temperature-time storage treatments, and for storage at −18° C until evaluated by the flavor panel. The entire food preparation and packaging operation was carried out in a sanitary manner, with workers using hats, masks covering mouth and nose, and rubber gloves.

Tests on Initial Blends

"Initial" blends are blends that were analyzed after being freshly prepared and blends that were stored at −18° C until analyses could be conducted. At this low temperature, blend characteristics remain essentially unchanged. Evaluations of the initial blends included proximate, amino acid, and sieve analyses; gruel consistencies; protein quality determined by animal assay; Hunter color values; chemical-stability indices, and microbiological analyses. Selected blends were evaluated by a flavor panel. Some tests were conducted only on blends without added antioxidants.

Generally, we used standard test methods. But there were several deviations from the standard 28-day AOAC (Association of Official Analytical Chemists 1975) procedure for the PER (protein efficiency ratio) evaluation of protein quality. PER is the gain in weight by a group of weanling rats divided by the weight of protein consumed (Jansen 1978). In our study, the diets, each containing a single commodity or particular food blend as the protein source, were calculated on a 10% protein level rather than on an isonitrogenous basis as in the AOAC procedure. This was done because the nitrogen factors of various blend components varied significantly from the 6.25 nitrogen factor assumed in the AOAC method. Nitrogen factors came from several sources: from Watt and Merrill (1963) for nonfat dry milk (6.38), peanut flour (5.46), cottonseed flour (5.30), soy flour (5.71), and corn meal (6.25); from manufacturer's information for whey protein concentrate (6.38); and from analytical data for lysine monohydrochloride (4.57). Composite nitrogen factors were calculated for each blend; these were based on the nitrogen factors of each ingredient and on the percentage of the protein content of each blend ingredient in the total formulation. These composite nitrogen factors were used to formulate diets on the 10% protein level for the animal assay. We also deviated from the AOAC PER procedure by using 5 animals rather than 10. The testing laboratory performing the assays has been routinely using 5 animals for some time for this test and has found only a small difference in the standard error of the mean between results for 5 and 10 animals. Still another change from the normal PER procedure was that two groups of animals in each run were fed standard diets

containing ANRC (Animal Nutrition Research Council) casein, the reference used as the basis of comparison with the test protein. For one group, the 10% ANRC casein level was computed (as in the AOAC procedure) on the basis of a nitrogen factor of 6.25; for the other group, the 10% ANRC casein level was computed on the basis of a nitrogen factor of 6.38 (Watt and Merrill 1963).

PER reflects the ability of a protein source to provide for the test animal's growth. We also used NPR (net protein ratio), another index of protein quality, which reflects the ability of the protein source to provide for both growth and maintenance.

Initial and stored blends were evaluated by a trained flavor panel. The selection of samples evaluated was based on microbiological criteria and on how much time the judges had available. The evaluations were conducted over a series of 11 sessions of the panel, with 5 to 12 judges participating in each session. The blends were evaluated as gruels at 10% solids; we prepared these by stirring the dry blend into boiling water and cooking for 1 minute (Bookwalter et al. 1971). Coded randomized samples were evaluated in comparison with a cornmeal standard for intensity of corn flavor, intensity of off-flavor, and overall flavor quality. Cornmeal was designated as the standard of comparison in this study because these corn-based gruels are intended to be nutritionally superior, partial replacements for the corn gruels that preschool children in certain developing parts of the world are already accustomed to.

Storage Tests

To assess their comparative storage stability, the blends, with and without antioxidants, were stored under the following temperature-time conditions: 25° C for 6 and 12 months, 37° C for 3 and 6 months, and 49° C for 1 and 2 months. Stored samples were evaluated for changes in color, lipid deterioration (free fatty acids and peroxide value), deterioration in protein quality (available lysine), microbial levels, and flavor.

Statistical Analysis

For Hunter color values, chemical stability indices (available lysine, free fatty acids, peroxide value), and microbial levels, we used analysis of variance to test for significance among the variations of storage temperature (3 levels), storage period (2 levels), added antioxidant (2 levels, with and without), and blend (11 blends). In dealing with storage time comparisons, absolute time intervals, such as zero (initial), 3 months, and 6 months, could not be used because storage intervals differed for each of the three storage temperatures. By use of the general designations of a "shorter" and "longer" period, the interaction of storage temperature and storage interval could be included in the analysis of variance. Initial values for these characteristics are presented separately. Also, because of the nature of microbial distributions, logarithmic transformations were made on the microbial levels in the statistical analyses for the purpose of stabilizing the variances (Snedecor and Cochrane 1980).

For statistical analysis of flavor panel scores, we compared four storage conditions: initial, 25° C for 12 months, 37° C for 6 months, and 49 °C for 2 months. Antioxidant level (with and without) and blends (10 in this case) were other sources of variation included. Only selected combinations of storage treatment, antioxidant level, and blend were rated by the flavor panel.

Where significant interactions among blends were indicated by analysis of variance, Duncan's new multiple-range test was used to detect significant differences among the blends. When significant differences involved quantitative sources of variation such as storage period, temperature-time combination, or antioxidant level, Duncan's test could not be validly applied. In such instances, we listed only means.

RESULTS AND DISCUSSION

Blend Ingredients

The characterization of the various blend ingredients by proximate analysis, sieve analysis, and levels of various micro-organisms is delineated in tables 1 and 2. All blend ingredients in table 2 reasonably conformed to the microbiological standards of the PAG guideline (Thatcher 1972).

Recently, World Hunger Program guidelines have been issued by the United Nations University, Tokyo, for peanut flour (Milner 1979), soy grits and flours (Milner 1980b), and cottonseed flours and related products (Milner 1980a). Both

peanut flours used in our study exceeded the stringent recommended maximum total plate count (20,000) of the new peanut guideline but met recommended requirements for *Escherichia coli, Salmonella,* aflatoxin, and freedom from pathogens at detectable levels. The soy flour used met the new soy guideline's requirements for total plate count, *E. coli, Salmonella,* aflatoxin, and freedom from pathogens at detectable levels. The glandless cottonseed flour exceeded the new cottonseed guideline's maximum for total plate count and the recommendations for negative coliforms but met the requirements for *E. coli, Salmonella,* staphylococci, aflatoxin, yeasts, and molds. The free gossypol content of the glandless cottonseed flour was 0.013%, which was considerably less than the 0.045% maximum level of the cottonseed guideline. Computer-determined ingredient compositions of blends without antioxidants, and limited by the specifications given above for nutrient and commodity levels are shown in table 3.

Chemical Composition of Blends

The proximate analyses, essential amino acid profiles, and chemical scores for the 11 experimental blends prepared without antioxidants are presented in table 3. The protein contents of the nine computer-formulated blends were slightly lower than the 20% level specified for the computer-formulation procedure. The lower than formulated protein contents may have been caused by some moisture absorbed during blending.

Table 2.—Microbiological analyses[1] of blend ingredients[2]

Ingredient	Total plate count (organisms/g)[3]	Total coliforms (MPN/g)	Fecal streptococci (organisms/g)	Thermophilic plate count (organisms/g)	Molds, (organisms/g)	Aflatoxin (p/b)
Cornmeal, processed, gelatinized.	2,500	9.1	<10	50	<10	<5
Cottonseed flour, glandless, defatted.	110,000	1100.0	280	45,000	20	<5
Peanut flour, solvent extracted.	54,000	43.0	1,800	16,000	400	<5
Peanut flour, full solvent extracted.	37,000	3.6	25	7,800	<10	<5
Soy flour, defatted, toasted.	10,000	0	1,200	650	<10	<5
Milk, nonfat, dry	3,500	0	1,100	40	<10	<20
Whey protein concentrate.	150	0	<10	80	<10	<20
Vitamin premix	100	0	<10	10	<10	<5
Mineral premix	700	0	<10	10	<10	<5
L-Lysine monohydrochloride.	<100	0	<10	10	<10	....
Cottonseed oil	<100	0	<10	10	<10	....
Soy oil	<10	0	<10	<10	<10	<5

[1]All tests except aflatoxin were done with AOAC (Association of Official Analytical Chemists 1975) procedures. Aflatoxin was determined by the minicolumn procedure (Holaday and Lansden 1975) on nondairy ingredients and by thin-layer chromatography (with the AOAC procedure) on dairy ingredients.

[2]No ingredient had fecal coliforms (including *E. coli*) or coagulase-positive staphylococci. All ingredients had fewer than 10 organisms/g of yeasts and *Clostridium perfringens.* All ingredients were negative for *Salmonella.* The PAG guideline No. 11 (Thatcher 1972) calls for rejection of a lot of dried milk if any sample exceeds 10 organisms/g of fecal coliforms or coagulase-positive staphylococci. But there may be basis for concern if any sample exceeds 0 for either. The PAG guideline No. 11 calls for rejection of the lot if any sample of protein concentrates from animal or vegetable sources, dried milk, or nuts tests positive for *Salmonella.*

[3]The PAG guideline No. 11 (Thatcher 1972) for total plate count (organisms/g) is: for cereal products and for protein concentrates from animal and vegetable sources, m=100,000, M=10 million; for dried milk, m=50,000, M=200,000; and for nuts, m=1 million, M=100 million. The symbol m is used for the number of organisms below which no basis for concern exists; M is used for the number of organisms that should not be exceeded by any sample—a greater number calls for rejection of the lot.

Table 3.—Composition and chemical constitution of initial blends without antioxidants

Blend	Composition[1] (%)							Proximate analysis					
	Corn-meal	Oilseed flour			Dairy product	Oil[2]	Lysine-HC1	H_2O (%)	Lipids (%)	Protein (%)	Ash (%)	Crude fiber (%)	CHO by difference (%)
		Soy	Peanut	Cotton-seed									
CSM (Public Law 480 blend).[4]	59.2	17.5	....	....	15.0	5.5	....	8.9	6.1	18.5	4.8	0.9	60.8
CP (pre) WL	56.2	....	19.6	....	15.0	6.3	0.1	9.3	6.9	19.2	4.6	.9	59.1
CP(full) WL	56.9	....	19.2	....	15.0	6.0	.1	9.2	6.9	19.5	5.0	.9	58.5
CCotW	55.7	....	....	20.4	15.0	6.1	....	9.6	6.3	19.3	4.5	1.0	59.3
CCot (3) W	55.4	5.2	....	15.6	15.0	6.1	....	9.3	7.0	19.9	5.1	1.0	57.7
CCotSW	55.0	10.6	....	10.6	15.0	6.1	....	9.6	6.8	19.1	5.1	.6	58.8
CCot (⅓) SW	54.6	5.4	16.1	15.0	6.1	....	9.6	7.0	19.3	5.1	.9	58.1	
CSW	54.2	21.9	....	....	15.0	6.1	....	9.6	6.8	19.1	5.1	.6	58.8
CS (Public Law 480 blend).[4]	69.7	22.0	....	....	....	5.5	....	9.7	6.9	15.4	4.7	1.0	62.3
CCot (soy) L	62.8	....	....	28.3	....	5.9	.2	9.3	7.1	19.8	4.3	1.0	58.5
CCot (cot) L	62.8	....	....	28.3	....	5.9	.2	9.2	6.8	19.2	4.6	1.3	58.9

Blend	Amino acid scores[3]							
	Iso-leucine	Leu-cine	Lysine	Methi-onine and cystine	Phenyl-alinine and tyrosine	Threo-nine	Trypto-phan	Valine
CSM (Public Law 480 blend).[4]	124	147	98	*86*	150	95	178	104
CP(pre)WL	110	143	*87*	88	146	95	250	95
CP(full)WL	102	139	*83*	89	140	93	179	89
CCot W	103	131	*88*	96	142	103	207	100
CCot(3)SW	106	127	92	*91*	134	101	217	98
CCotSW	114	136	98	*96*	140	106	251	101
CCot(⅓)SW	110	137	100	*83*	135	105	228	96
CSW	118	139	102	*79*	136	109	224	99
CS(Public Law 480 blend).[4]	109	143	*84*	96	144	89	181	95

See footnotes at end of table.

Table 3.—Composition and chemical constitution of initial blends without antioxidants—Continued

	Amino acid scores[3]							
	Iso-leucine	Leu-cine	Lysine	Methi-onine and cystine	Phenyl-alinine and tyrosine	Threo-mine	Trypto-phan	Valine
CCot(soy)L	*81*	125	86	86	149	86	212	90
CCot(cot)L	91	126	90	94	154	*85*	177	96

[1]Each blend also included mineral (1.7%) and vitamin (1.1%) premixes as described in U.S. Agricultural Stabilization and Conservation Service (1979).

[2]Soy oil was used except in CCot(cot)L, which contained cottonseed oil.

[3]The amino acid scores were computed according to the definition of an equation published by FAO/WHO (Joint FAO/WHO *Ad Hoc* Expert Committee 1973) except that, for each protein mixture, actual nitrogen factors (Watts and Merrill 1963) for component proteins were used instead of the conventional nitrogen factor of 6.25. For each blend, the chemical score (the lowest amino acid score) is given in italics.

[4]The actual Public Law 480 blends contain added antioxidants.

From a practical production standpoint, the protein contents were considered to be adequately close to the goal level. It is noted that the protein contents for the Public Law 480 blends, CSM and especially CS, were even lower than those for the computer-formulated blends. One reason for this is that these blends are formulated by proportions of commodities that vary somewhat in nutrient levels from batch to batch. Another reason is that CS is formulated to contain a lower protein than the general USDA goal level of 20%.

Amino acid scores, chemical scores, and the limiting amino acids listed in table 3 make up profiles of the essential amino acids for these blends. A nonessential amino acid reduces the requirement of an essential one in two instances. Cystine reduces the need for methionine, and tyrosine reduces the need for phenylalanine. So the cystine contribution in a food commodity is added to that of methionine, and the tyrosine contribution is added to that of phenylalanine. The actual chemical scores (table 3) varied by an average of 6.1% from computer-predicted chemical scores, which were based on the proximate and amino acid analyses of blend ingredients. The difference between predicted and actual was probably the result of components being incompletely mixed and of lack of better precision in analytical procedures.

Texture of Blends

Sieve analyses and consistencies of cooked gruels for the 11 initial blends without antioxidants are given in table 4. All blends containing a dairy product met the requirements of sieve analysis and gruel consistency of the corn-soy-milk specification (U.S. Agricultural Stabilization and Conservation Service 1979). Those blends not containing a dairy product met the requirements for sieve analysis and gruel consistency of the corn-soy specification (U.S. Agricultural Stabilization and Conservation Service 1978a).

Protein Quality

Nitrogen factors, PER, NPR, and nitrogen digestibilities for the 11 blends without antioxidants and for the two experimental peanut flours are presented in table 5. Overall, CCot(soy)L and CCot(Cot)L compared particularly well in adjusted PER and NPR values to CSM and CS. The

Table 4.—Sieve analyses and gruel consistencies of initial blends without antioxidants

Blend	Percentage of blend passing through U.S. sieve No.[1]—			Bostwick reading[2]	
	6	30	60	Cooked blend	Uncooked blend
	Blends containing dairy product				
Corn-soy-milk specification[3]	≥99.0	≥92.0	≥60.0	10-22	≥20
CSM (Public Law 480 blend)[4]	100.0	79.8	26.6	19.5	14.0
CP(pre)WL	100.0	82.1	47.7	18.5	10.5
CP(full)WL	100.0	82.3	44.2	13.0	19.5
CCotW	100.0	84.0	52.8	15.0	14.0
CCot(3)SW	100.0	84.4	53.1	15.5	20.0
CCotSW	100.0	84.5	53.2	20.0	19.5
CCot(⅓)SW	100.0	83.6	53.2	20.5	19.0
CSW[5]	100.0	81.8	53.0	20.5	19.5
	Blends without dairy product				
Corn-soy specification[6]	≥99.0	≥92.0	≥57.0	9-21	≥20
CS[7] (Public Law 480 blend)[4]	100.0	80.0	40.1	14.0	14.5
CCot(soy)L	100.0	76.2	33.7	16.0	20.0
CCot(cot)L	100.0	77.8	34.2	12.5	17.0

[1]The standard ASTM (American Society for Testing and Materials Committee E-29 1972) procedure was followed for sieve analyses. Samples were split with a Jones-type sample splitter. A mechanical sieve shaker with tapper (Ro-Tap testing sieve shaker, W. S. Tyler Co.) was used in the analyses with U.S. standard sieves. Optimum sample weight was found to be 50 and optimum shaking time, 15 minutes.

[2]The consistency of gruels prepared from the dry blends was measured with the Bostwick consistometer (Bookwalter et al. 1968). The consistency of those containing dairy product was measured according to the corn-soy-milk specification of the U.S. Agricultural Stabilization and Conservation Service (1979). The consistency of those without dairy product was measured according to the corn-soy specification of the U.S. Agricultural Stabilization and Conservation Service (1978a).

[3]U.S. Agricultural Stabilization and Conservation Service (1979).

[4]The actual Public Law 480 blends contain added antioxidants.

[5]The moisture content of CSW exceeded the maximum set by the corn-soy-milk specification by 0.1%.

[6]U.S. Agricultural Stabilization and Conservation Service (1978a).

[7]The moisture content of CS exceeded the corn-soy specification by 0.1%.

blends containing peanut flour were the least impressive, and other blends were intermediate in this comparison.

Relative differences in NPR values among blends generally reflected relative differences in PER values (table 5). But *F*-values obtained from the analyses of variance of the data for each of the three animal runs are considerably larger for PER than they are for NPR. The *F*-ratios for animal group 1 were 34.07 for PER and 20.45 for NPR; for animal group 2, they were 65.16 for PER and 19.09 for NPR; for animal group 3, they were 11.21 for PER and 6.07 for NPR. The *F*-ratio is the ratio of variance between sets of animals on the same protein source to the experimental error term. For each of the three animal runs represented, $P<0.01$ when $F>3.90$. The higher *F*-values for PER indicate that it was more sensitive than NPR in detecting differences among samples. The difference in sensitivity can also be seen in table 5 by comparing the results of Duncan's new multiple-range test on the PER and NPR values. Such information is important in selection of an animal assay procedure that is best able to discriminate between blends of different protein quality. It should be pointed out, however, that there are other workers who have found NPR to be more suitable than PER for the

Table 5.—Animal assays of initial blends[1] without antioxidants

Animal group[2] run and dietary source of protein[3]	Nitrogen factor[4] (n.f.)	PER[5] values			NPR[6] values			Nitrogen digestibility[7] (%)
		Actual[8] (mean ±standard error	Adjusted to ANRC casein		Actual[8] (mean ±standard error	Relative to ANRC casein		
			(n.f.= 6.25)	(n.f.= 6.38)		(n.f.= 6.25)	(n.f.= 6.38)	
Animal group 1:								
ANRC casein control (n.f.=6.25).	6.25	3.49Aab±0.10	2.50	.....	4.52Aab±0.13	100	.....	93
ANRC casein control (n.f.=6.38).	6.38	3.52Aa±0.10	.....	2.50	4.63Ad±0.12	.....	100	94
CSM (Public Law 480 blend)[9]	6.03	3.26Ab±0.02	2.34	2.32	4.29Ab±0.02	95	93	85
CP(pre)WL	5.83	2.44Cd±0.14	1.75	1.73	3.55Bc±0.14	79	77	87
CP(full)WL	5.83	2.53Cd±0.03	1.81	1.80	3.55Bc±0.01	79	77	87
CCotW	5.74	2.90Bc±0.03	2.08	2.06	3.84Bc±0.10	85	83	85
Animal group 2:								
ANRC casein control	6.25	3.16ABab±0.10	2.50	.....	4.01Aa±0.13	100	.....	93
ANRC casein control (n.f.=6.38).	6.38	3.33Aa±0.07	.....	2.50	4.15Aa±0.04	.....	100	93
CS(Public Law 480 blend)[9]	5.89	2.95Cc±0.05	2.33	2.21	4.03Aa±0.07	100	97	83
CCot(soy)L	5.52	3.03BCbc±0.04b	2.40	2.27	4.05Aa±0.07	101	98	87
CCot(cot)L	5.52	3.23ABa±0.04	2.56	2.42	4.17Aa±0.08	104	100	88
Peanut flour (prepressed, solvent extracted).	5.46	2.25Dd±0.06	1.78	1.69	3.32Bb±0.12	83	80	91
Peanut flour (full solvent extracted).	5.46	2.12Dd±0.04	1.68	1.59	3.28Bb±0.06	82	79	91
Animal group 3:								
ANRC casein control (n.f.=6.25).	6.25	3.39Aa±0.11	2.50	.....	4.49Aa±0.16	100	.....	93
ANRC casein control (n.f.=6.38).	6.38	3.14ABb±0.07	.....	2.50	4.11ABb±0.10	.....	100	93
CCot(3)SW	5.79	2.86BCc±0.04	2.11	2.28	3.87Bb±0.10	86	94	86
CCotSW	5.86	2.88BCc±0.09	2.12	2.29	3.98Bb±0.11	89	97	84
CCot(⅓)SW	5.91	2.85BCc±0.03	2.10	2.27	3.91Bb±0.02	87	95	84
CSW	5.98	2.73Cc±0.07	2.01	2.17	3.81Bb±0.07	85	93	87

[1]Includes also peanut flour samples used in peanut-containing blends.

[2]Male, Sprague-Dawley rats; five rats per protein source in each animal run group; initial age of rats: 21 days; initial weight: 54 g for group 1, 55 g for group 2, and 54 g for group 3.

[3]Diets contained 10% protein.

[4]The nitrogen factor of each blend was calculated from actual nitrogen factors of blend components.

[5]PER (protein efficiency ratio)=weight gain/protein intake (28 days).

[6]NPR (net protein ratio)=weight gain over animals on a protein-free diet/protein intake (13 days) (Jansen 1978).

foodstuffs they examined (Pellett and Young 1980).

A key question in the use of the computer optimization procedure to obtain the best blend chemical score is whether the best protein quality by animal assay is also obtained. By including Food and Agriculture Organization (FAO) data for 13 other foodstuffs along with the data for the 11 experimental corn-based blends of this study, we found significance at $P<0.01$ (simple correlation coefficient, $r=0.83$; actual nitrogen values) between unadjusted PER and chemical score over the broad chemical score range of 49 to 100 (fig. 1). The use of actual nitrogen factors for calculation of chemical score resulted in a higher correlation coefficient ($r=0.83$ versus $r=0.76$) than that obtained by calculation of chemical score in the conventional way, which is based on using a nitrogen factor of 6.25 for all proteins. Protein$\times 100 \div$*nitrogen digestibility* resulted in a lower correlation with chemical score ($r=0.75$ versus $r=0.83$; actual nitrogen factors) than that obtained by using PER alone. This finding was contrary to expectation, since undigested protein is not usable by the animal body. The reason for not finding better correlation when digestibility was taken into account is not clear. Despite the differences in correlation coefficients cited in these two comparisons, all the correlations were significant at the $P<0.01$ level. Although the combined experimental corn blends and FAO data in figure 1 show significant correlation ($P<0.01$) over the broad chemical score range represented, significant correlation of PER with chemical score was not found over the rather narrow chemical score range (79 to 96) represented by the group of 11 experimental corn-based blends alone. Table 3 shows that several of these blends had numerically close chemical scores. Lack of significant correlation over the range represented by these closely spaced data points may be partly explained by factors such as disproportions in amino acid patterns, variability in test animals, and lack of precision in amino acid analysis. The precision of the gas chromatographic method is of the order of $\pm 10\%$ of aliquots of the same sample for some of the amino acids. Harper and Benevenga (1970) have pointed out that a surplus of amino acids, other than of the most limiting one on which chemical score is based, can depress growth and food intake in animals (and thereby affect PER). The influence of animal variability is seen in table 5, which reveals ranges in PER casein control

[7]Nitrogen digestibility=[(N intake−fecal N)/N intake]×100. Pooled data, from 6th through 13th test days.

[8]Within a group and column, means followed by the same uppercase letter are not significantly different when $P<0.01$; those followed by the same lowercase letter are not significantly different when $P<0.05$ (by Duncan's new multiple-range test). Statistical comparisons could not validly be made between runs. One of the 4 NPR values for CP(full) WL in animal group 1 and for CS in animal group 2 was outside the $P<0.05$ limit and was not included in the mean.

[9]The actual Public Law 480 blends contain added antioxidants.

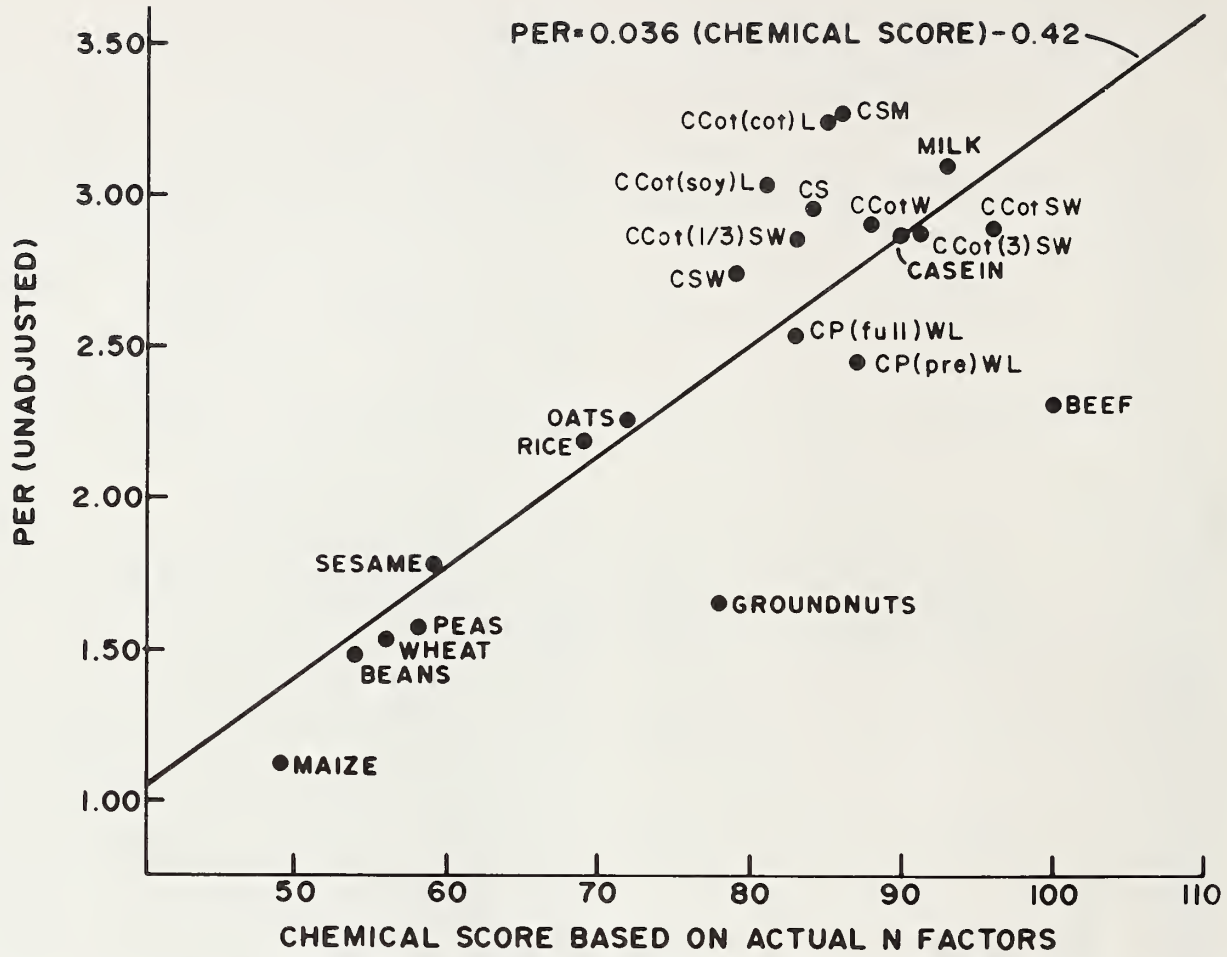

FIGURE 1.—Linear regression of PER on chemical score for various foods and the corn-based blends. For foods other than the corn-based blends, PER's and amino acid compositions for calculation of chemical scores were obtained from the Food and Agriculture Organization (1970). Chemical scores were computed according to the definition of the Joint FAO/WHO *Ad Hoc* Expert Committee (1973), except that actual factors for the individual commodity proteins were used instead of the conventional nitrogen factor of 6.25. Simple correlation coefficients for PER versus chemical score using these combined data were 0.83 when based on actual nitrogen factors and 0.76 when based on milligrams of amino acid per gram times nitrogen. For PER (100/nitrogen digestibility) versus chemical score, they were 0.75 when based on actual nitrogen factors and 0.66 when based on milligrams of amino acid per gram times nitrogen. All correlations are significant when $P < 0.01$.

group means for the three animal runs of 3.16 to 3.49 for the 6.25 nitrogen factor groups and of 3.14 to 3.52 for the 6.38 nitrogen factor groups. It should be pointed out that there are other animal assay procedures, such as RPV (relative protein value), that, although more expensive, provide responses more proportional to the quality of the protein than does PER (Pellet and Young 1980). But the PER procedure is used for regulatory purposes in the United States and Canada, so it is necessarily of importance to some groups concerned with protein quality. Based on the experimental findings described above, we suggest that more test animals and more amino acid replicates than customary should be used in attempts to correlate PER with chemical score.

Storage Behavior

Color

In initial blends, as expected, a higher percentage of cottonseed in a blend resulted in increasing greenness, especially in the two corn-cottonseed-lysine-monohydrochloride blends (table 6). Increasing the quantity of soy increased yellowness, especially in CS. All blends had a generally satisfactory appearance.

As storage temperature increased from 25° C to

Table 6.—Color values and chemical-stability indices of initial blends

Blend	Added antioxidants?	Hunter color values[1]			Available lysine[2] percentage of protein	Free fatty acid[3] (as percentage of oleic in total lipid)	Peroxide value[4] (meq/kg fat)
		L	*a*	*b*			
CSM (Public Law 480 blend).[5]	No	78.3	−0.7	24.4	4.1	1.4	1.09
	Yes	78.4	− .9	24.5	3.8	.9	.95
CP(pre)WL	No	78.5	− .7	24.5	3.9	1.6	2.06
	Yes	78.3	− .6	24.8	4.1	2.0	1.11
CP(full)WL	No	83.7	−1.4	19.7	4.1	2.4	16.71
	Yes	83.2	−1.4	19.8	4.0	2.0	4.69
CSW	No	82.9	−2.2	19.3	4.1	2.2	2.06
	Yes	82.6	−2.2	19.6	4.2	3.8	.89
CCot(3)SW	No	81.8	−1.9	20.2	4.1	3.2	.30
	Yes	82.2	−1.9	20.3	4.5	2.9	.80
CCotSW	No	81.1	−1.7	21.0	4.5	3.7	.63
	Yes	80.6	−1.7	21.2	4.1	1.8	1.03
CCot(⅓)SW	No.	79.9	−1.1	22.3	4.0	4.8	2.02
	Yes	79.8	−1.2	22.6	3.9	3.9	1.15
CSW	No	79.0	− .8	23.7	3.8	1.6	1.36
	Yes	78.9	− .7	23.5	3.9	2.1	.76
CS (Public Law 480 blend).[5]	No	76.3	− .2	26.6	5.6	.8	.58
	Yes	76.6	− .4	26.0	4.6	.8	1.47
CCot(soy)L	No	82.4	−2.5	18.5	4.0	6.3	.55
	Yes	82.2	−2.2	18.5	4.3	6.4	1.43
CCot(cot)L	No	82.1	−2.2	18.7	4.2	6.2	3.22
	Yes	82.3	−2.2	18.7	4.1	6.1	.95

[1]Determination of reflectance color *L*-, *a*-, and *b*-values was done on 10 g of blend with a Hunter digital color difference meter, model D25D2A. *L* (lightness), *a* (+red,−green), *b* (+yellow,−blue).

[2]Method of Rao et al. (1963).

[3]Modified from method of American Oil Chemists' Society (1979).

[4]Association of Official Analytical Chemists (1975).

[5]The actual Public Law 480 blends contain added antioxidants.

37° C to 49° C, the blends usually became darker, more red, and slightly more yellow (tables 7,8). The degree of change in the three color values varied with increasing temperature differentially according to blend. Of special note is that those blends without nonfat dry milk or whey protein concentrate exhibited the greatest color stability (that is, the *L*-, *a*-, and *b*-values changed the least with increasing storage temperature). Apparently, then, the dairy ingredient is the one most liable to change color as storage temperature increases. Analysis of variance showed significant differences in Hunter *b*-values among storage temperatures, depending either on storage interval or on the level of added antioxidants. There is little absolute difference in *b*-values, even though the differences are statistically significant.

Increasing storage period generally resulted in blends becoming darker and more red (table 8). Some blends became a little more yellow, other blends a little less yellow as storage time increased. Analysis of variance (table 7) showed significant differences ($P<0.01$) in the influence of storage period on color values, depending on the blend. As with storage temperature, *L*- and *a*-values changed least in the blends without a dairy product as storage time increased. But *b*-values in the blends without dairy products changed most with increased storage; these changes were moderate in terms of absolute units of *b*-value.

Hunter *b*-values differed significantly among blends ($P<0.01$), depending on whether antioxidants had been added or not. But the *b*-value comparisons listed in table 8 show that the differences between samples, with and without added antioxidants, did not vary much among blends in terms of absolute *b*-value units. So the differences, although significant, may not be of practical importance.

Table 7.—Analyses of variance of color values, chemical-stability indices, and microbial levels for stored blends

Sources of variation	Significance of differences[1]										
	Hunter color values[2]			Available lysine	Free fatty acid	Peroxide value	Log_{10} (microbial level[2])				
	L	*a*	*b*				Total plate count (organisms/g)	Total coliforms (MPN/g)	Fecal streptococci (organisms/g)	Thermophilic plate count (organisms/g)	Mold count (organisms/g)
Among storage-temperature treatments (25° C, 37° C, 49° C).	*	**	**	NS	**	**	**	*	**	**	**
Dependent on whether exposed to shorter or longer storage time.[3]	NS	NS	**	NS	NS	**	**	NS	NS	**	**
Dependent on whether antioxidants are added or not.	NS	NS	**	NS	NS	**	NS	NS	NS	NS	NS
Dependent on blend	**	**	**	NS	**	**	NS	NS	*	NS	*
Between shorter and longer storage times.[3]	**	**	**	*	**	NS	NS	NS	NS	NS	NS
Dependent on whether antioxidants are added or not.	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Dependent on blend	**	**	**	NS	*	*	NS	NS	NS	*	NS
Between no added antioxidants versus added antioxidants.	NS	NS	*	**	NS	**	NS	NS	NS	NS	NS
Dependent on the blend	NS	NS	**	**	NS	**	NS	NS	NS	NS	NS

[1]Estimates of statistical differences are based on using, as the error term, the mean square of the interaction of the temperature storage treatment by the storage time exposure by the antioxidant level of the blend. (*=$P<0.05$; **=$P<0.01$; NS=significance tested for but not found).

[2]Fecal coliforms, coagulase-positive staphylococci, yeasts, *Salmonella, Clostridium perfringens,* and aflatoxin remained below detectable limits in all samples.

[3]Shorter storage times: 25° C for 6 mo; 37° C for 3 mo; 49° C for 1 mo; longer storage times: 25° C for 12 mo; 37° C for 6 mo; 49° C for 2 mo.

Table 8.—Hunter color values[1] reflecting significant[2] interactions reported in table 7[3]

Blend	Interaction of blend and storage temperature								
	L-value at—			*a*-value at—			*b*-value at—		
	25° C	37° C	49° C	25° C	37° C	49° C	25° C	37° C	49° C
CSM (Public Law 480 blend)[4]	78.85ef	76.87d	69.74f	0.05ab	0.08cd	3.69c	23.23c	24.07c	26.50a
CP(pre)WL	78.33f	72.29g	66.10g	.05ab	2.43a	5.14b	23.63b	25.79a	25.91a
CP(full)WL	83.87a	79.86b	73.51d	−.41cd	.27cd	2.94d	18.70g	21.48f	23.71f
CCotW	82.58b	79.58b	75.01b	−.55d	−.75e	1.51f	18.60g	20.52g	22.30h
CCot(3)SW	81.57c	77.76c	73.56d	−.43cd	−.03cd	2.06e	19.61f	21.69e	23.46g
CCotSW	80.56d	77.02d	72.06e	−.03bc	.30c	2.73d	20.76e	22.79d	24.29e
CCot(⅓)SW	79.43e	74.64f	69.46f	−.03bc	1.38b	3.89c	22.10d	24.43b	25.26c
CSW	78.61f	72.24g	65.11h	.45a	2.73a	5.87a	23.20c	25.69a	26.09b
CS (Public Law 480 blend)[4]	76.48g	75.91e	74.21c	.31ab	−.10d	.77g	25.45a	24.43b	24.81d
CCot(soy)L	82.42b	81.89a	81.04a	−.76d	−2.07f	−.84h	17.64h	17.74h	18.26i
CCot(cot)L	82.35b	81.98a	81.29a	−.70d	−2.08f	−.85h	17.88h	17.73h	18.09i

Blend	Interaction of blend and storage period[5]						Interaction of blend and antioxidant level	
	L-value for—		*a*-value for—		*b*-value for—		*b*-value when—	
	Shorter	Longer	Shorter	Longer	Shorter	Longer	None added	Added
CSM (Public Law 480 blend)[4]	76.22e	73.14f	0.59d	2.33d	24.49c	25.01a	24.63c	24.82c
CP(pre)WL	72.88g	70.26g	1.98b	3.70b	25.39a	25.07a	25.07b	25.42a
CP(full)WL	79.80b	77.26c	0.46d	1.77e	21.27g	21.85e	21.44g	21.62g
CCotW	79.58b	77.72b	−.38f	0.73g	20.66h	20.62f	20.60h	20.68h
CCot(3)SW	78.20c	76.16d	.13e	1.22f	21.63f	21.94e	21.76f	21.77g
CCotSW	77.18d	74.99e	.58d	1.70e	22.70e	22.89d	22.65e	22.91f
CCot(⅓)SW	75.23f	72.78f	1.19c	2.74c	23.98d	24.15c	24.11d	24.01e
CSW	72.84g	69.64h	2.43a	4.24a	25.18b	25.14a	25.26a	25.05b
CS (Public Law 480 blend)[4]	75.59f	77.28e	.03e	.69g	25.23ab	24.38b	25.06b	24.63d
CCot(soy)L	81.78a	81.67a	−1.40g	−1.11h	18.22i	17.52g	17.74i	18.06i
CCot(cot)L	81.77a	81.91a	−1.33g	−1.18h	18.32i	17.40g	17.73i	18.04i

[1]Determination of reflectance color *L*-, *a*-, and *b*-values was done on 10 g of blend with a Hunter color difference meter, model D25D2A. *L* (lightness), *a* (+red,−green), *b* (+yellow,−blue). Each value is a mean obtained by averaging over the other variables. For example, the *L*-value at 25° C for CSM was obtained by averaging values over the different storage periods and antioxidant levels.

[2]Within a group and column, means followed by the same letter are not significantly different ($P<0.05$) by Duncan's new multiple-range test.

[3]With storage period as the source of variation, *b*-values for interactions of blend and storage temperature were: 25° C, 21.29; 37° C, 22.30; and 49° C, 23.49 for shorter periods and 25° C, 20.35; 37° C, 22.39; and 49° C, 23.32 for longer. With antioxidant level as the source of variation, they were: 25° C, 20.86; 37° C, 22.38; and 49° C, 23.49 when no antioxidants were added and 25° C, 21.10; 37° C, 22.31; and 49° C, 23.55 when they were added. These data could not be subjected to Duncan's new multiple-range test because the source of variation is quantitative.

[4]The actual Public Law 480 blends contain added antioxidants.

[5]Shorter storage periods: 25° C, 6 months; 37° C, 3 months; and 49° C, 1 month. Longer storage periods: 25° C, 12 months; 37° C, 6 months; and 49° C, 2 months.

Available lysine

Available lysine has been used in this study as an index of protein deterioration via reactions, such as nonenzymatic browning, that tie up the ϵ-amino group of the lysine molecule. Besides reducing sugars, the autoxidation of fats or fatty acids may yield carbonyl groups for a Maillard-type reaction with the ϵ-amino group (Anglemier and Montgomery 1976). So antioxidants can influence available lysine values.

Available lysine values in fresh blends are shown in table 6. Analysis of variance (table 7) showed no significant differences among temperature treatments, but available lysine did decrease significantly ($P<0.05$) with increasing storage time, though the difference was small. Means listed in table 9 indicate diverse differences among blends with regard to whether there was a significant influence ($P<0.05$) of added antioxidants on lysine value (table 7). In general, the differences between a blend with added antioxidants and the same blend without were small. CSM and CS had appreciably larger differences than the others. The larger effects for these two blends are apparently not related to soy content. Overall, data on available lysine did not reflect any substantial deterioration in proteins. Also, the addition of lysine monohydrochloride to certain blends did not appear to particularly influence the rate of loss of lysine in those blends.

Free fatty acids

Free fatty acids may result from lipid hydrolysis caused by enzymes, thermal stress, and chemical action, and may influence the flavor, texture, and other properties of food (Dugan 1976). Free fatty acids released by lipid hydrolysis may be further degraded into possibly undesirable chemical substances in foods.

Free fatty acid values in initial blends are shown in table 6. Analysis of variance (table 7) showed that temperature affected free fatty acid values significantly ($P<0.01$), dependent on the blend. The free fatty acid content of the blends depended on the cottonseed content, and cottonseed-containing blends declined in free fatty acids with increasing temperature (table 9). The blends without cottonseed flour changed little if at all in free fatty acids with increasing storage temperature.

Generally, storage produced large increases in free fatty acids in blends containing cottonseed flour, especially at the lowest storage temperature (table 9). Free fatty acids increased significantly ($P<0.01$) with increasing storage time, with a significant ($P<0.05$) dependence on blend (table 7). Storage times longer than those used in this study may possibly result in changes in organoleptic quality of cottonseed-containing blends. As would be expected, antioxidant level did not significantly affect free fatty acid content of the blends (table 7).

Peroxide values

The peroxide value is a measurement of the state of oxidation of lipids to the point where decomposition of hydroperoxides starts. Beyond this point, peroxide value decreases, although the effect of deterioration of product flavor becomes more marked (Dugan 1976).

Peroxide value on initial blends are shown in table 6. Storage temperature significantly ($P<0.01$) affected peroxide value (table 7), with a significant ($P<0.01$) dependence on blend. The peroxide values for the two blends containing peanut flour were much higher than those for the other blends at 25° C (table 9). But the differences essentially disappeared at 37° C, most likely because of peroxide decomposition in the peanut blends. At 49° C, some significant differences appeared among peroxide values of certain blends, but the differences were much smaller than those found at 25° C. Analysis of variance also showed that temperature significantly ($P<0.01$) affected peroxide value independent of storage time and antioxidant level. Mean values showing these effects are presented in table 9. At 25° C, peroxide value was greater after 12 months of storage than it was after 6; at 37° C, it was less at 6 months than it was at 3; at 49° C, there was little difference between the 1- and 2- month values. At 25° C, peroxide value was much lower in blends with added antioxidants than in those without, but no differences were evident at 37° C or at 49° C. Apparently, the influence of storage temperature on the effects of storage period and antioxidant level reflect the more rapid rate of degradation of hydroperoxides with increasing temperature.

Analysis of variance showed that storage time ($P<0.05$) and the addition of antioxidants ($P<0.01$) significantly affected peroxide level

Table 9.—Chemical stability indices[1] reflecting significant[2] interactions reported in table 7[3]

Blend	Interaction of blend and storage temperature						Interaction of blend and storage period[4]				Interaction of blend and antioxidant level			
	Free fatty acid (as percentage of oleic in total lipid) for—			Peroxide value (meq/kg fat) at—			Free fatty acid (as percentage of oleic in total lipid) for—		Peroxide value (meq/kg fat) for—		Available lysine (percentage of protein) when—		Peroxide value (meq/kg fat) when	
	25° C	37° C	49° C	25° C	37° C	49° C	Shorter	Longer	Shorter	Longer	None added	Added	None added	Added
CS (Public Law 480 blend).[5]	1.0f	1.0d	1.0d	1.61c	3.85a	2.20b	0.9d	1.1e	2.29c	2.82c	4.4b	4.0bc	2.7c	2.4bc
CP(pre)WL	1.4f	2.6d	2.4d	28.29b	5.27a	4.97ab	2.2d	2.1e	10.12b	15.57b	4.1cde	4.0bc	19.9b	5.8b
CP(full)WL	1.1f	4.5d	1.1d	58.45a	4.52a	2.69ab	1.0d	3.4e	19.38a	24.39a	3.9ef	3.9c	28.2a	15.6a
CCotW	22.9bc	15.5b	10.9b	.90c	3.15a	3.89ab	13.3b	19.6b	3.23c	2.06c	4.1cde	4.0bc	2.8c	2.5bc
CCot(3)SW	20.9cd	17.3b	13.5b	1.60c	4.45a	7.19a	16.1b	18.3b	6.01c	2.82c	4.0def	4.2b	3.2c	5.7bc
CCotSW	17.2d	13.7bc	10.7b	2.05c	5.58a	2.98ab	12.9b	14.8c	3.50c	2.24c	4.3bc	4.1b	2.7c	3.1bc
CCot(⅓)SW	12.8e	10.0c	6.5c	.95c	3.33a	3.04ab	8.1c	11.4d	2.59c	2.29c	4.1cd	4.1b	3.1c	1.7c
CSW	1.6f	2.8d	1.2d	2.95c	2.87a	1.68b	2.7d	1.1e	2.27c	2.73c	3.8f	3.8c	2.9c	2.1bc
CS (Public Law 480 blend).[5]	.8f	.9d	1.2d	2.03c	2.55a	2.04b	1.0d	1.0e	2.44c	1.98c	5.4a	5.0a	2.3c	2.1bc
CCot(soy)L	27.8a	24.5a	21.2a	.33c	3.18a	3.41ab	22.6a	26.4a	2.78c	1.83c	3.9ef	4.0bc	2.3c	2.4bc
CCot(cot)L	26.6ab	24.6a	22.7a	.86c	4.03a	5.15ab	22.1a	27.2a	4.32c	2.37c	4.2bcd	4.1b	3.7c	3.0bc

[1]Each value is a mean obtained by averaging for that chemical index over the other variables. For example, the percentage of free fatty acid for CSM at 25° C was obtained by averaging values over the different storage periods and antioxidant levels.

[2]Within a group and column, means followed by the same letter are not significantly different ($P<0.05$) by Duncan's new multiple-range test.

[3]With storage period as the source of results for variation, peroxide values (in meq/kg fat) for interactions of blend and storage temperature were: 25° C, 7.69; 37° C, 4.91, and 49° C, 3.47 for shorter storage periods and 25° C, 10.50; 37° C, 2.50; and 49° C, 3.66 for longer. With antioxidant level as the source of variation they were: 25°C, 12.62; 37° C, 4.12; and 49° C, 3.35 when no antioxidants were added and 25° C, 5.56; 37° C, 3.29; and 49° C, 3.79 when they were added. These data could not be subjected to Duncan's new-multiple range test because the source of variation is quantitative.

[4]Shorter storage periods: 25° C, 6 months; 37° C, 3 months, and 49° C, 1 month. Longer storage periods: 25° C, 12 months; 37° C, 6 months; and 49° C, 2 months.

[5]The actual Public Law 480 blends contain added antioxidants.

Table 10.—Microbiological analyses[1] of initial blends

Blend	Added antioxidants	Total place count (organisms/g)[3]	Total coliforms (MPN/g)	Fecal streptococci (organisms/g)	Thermophilic plate count (organisms/g)	Molds (organisms/g)	Aflatoxin (p/b)
CSM (Public Law 480 blend).	No	1,100	....	150	450	<10	<20
	Yes	450	....	350	900	30	<20
CP(pre)WL	No	4,000	43.0	16	400	55	<20
	Yes	11,000	23.0	12	450	200	<20
CP(full)WL	No	14,000	9.1	<10	100	<10	<20
	Yes	1,300	3.6	35	150	<10	<20
CCotW	No	16,000	23.0	<10	6,600	15	<20
	Yes	8,000	460.0	<10	3.200	10	<20
CCot(3)SW	No	11,000	9.1	<10	700	20	<20
	Yes	8,000	93.0	<10	1,200	10	<20
CCotSW	No	23,000	240.0	55	550	30	<20
	Yes	4,000	240.0	<10	400	15	<20
CCot(⅓)SW	No	4,000	93.0	<10	100	25	<20
	Yes	5,500	23.0	<10	400	35	<20
CSW	No	750	9.1	<10	600	<10	<20
	Yes	1,100	9.1	<10	450	10	<20
CS (Public Law 480 blend).[4]	No	10,000	23.0	10	250	<10	<5
	Yes	200	9.1	10	350	<10	<5
CCot(soy)L	No	13,000	1,500.0	<10	1,800	30	<5
	Yes	19,000	240.0	<10	1,800	15	<5
CCot(cot)L	No	3,500	460.0	<10	2,600	150	<5
	Yes	20,000	23.0	<10	2,000	25	<5

[1]All tests except aflatoxin were done with AOAC (Association of Official Analytical Chemists 1975) procedures. Aflatoxin was determined by the minicolumn procedure (Holaday and Lansden 1975) on blends without dairy ingredients and by thin-layer chromatography (with the AOAC procedure) on blends with dairy ingredients.

[2]No blend had fecal coliforms (including *E. coli*) or coagulase-positive staphylococci. All blends had fewer than 10 organisms/g of yeasts and *Clostridium perfringens*. All blends were negative for *Salmonella*. The PAG guideline No. 11 (Thatcher 1972) calls for rejection of the lot if any sample exceeds 10 organisms/g of fecal coliforms or coagulase-positive streptococci. The CSM commodity specification (U.S. Agricultural and Stabilization Service 1979) stipulates that fecal coliforms must be negative and that aflatoxin must not exceed 20 p/b. Both the PAG guideline No. 11 and the CSM commodity specification stipulate that *Salmonella* must be negative.

[3]The PAG guideline No. 11 (Thatcher 1972) calls for rejection of the lot if total plate count exceeds 20,000 organisms/g and states that a basis for concern exists if it exceeds 10,000 organisms/g. The CSM commodity specification (U.S. Agricultural Stabilization and Conservation Service 1979) stipulates that total plate count must not exceed 50,000 organisms/g.

[4]The actual Public Law 480 blends contain added antioxidants.

(table 7). A longer storage interval resulted in a marked increase in average absolute peroxide values for each of the two peanut-containing blends (table 9), whereas for the other blends there was a decrease or only a small increase. These mean peroxide values have been averaged over the remaining experimental variables, namely temperature and antioxidant level. In the case of the two peanut-containing blends, there were large magnitude decreases in peroxide value when antioxidants were added. For other blends, the effects of added antioxidants were much less.

Defatted glandless cottonseed, soy, and peanut flours are all known to contain natural antioxidants (Ziprin et al. 1981). In our study, however, peroxide values in the blends containing peanut flours from different sources indicated much greater lipid oxidation than did blends containing cottonseed, soy, or both.

Microbial levels

The initial blends with and without antioxidants, conformed to the corn-soy-milk specification (U.S. Agricultural Stabilization and Conservation Service 1979) for total plate count, fecal coliforms, *Salmonella,* and aflatoxin as shown in table 10. The initial blends were also in reasonable conformation to the requirements of

Table 11.—Logarithms$_{10}$ of microbial counts[1] reflecting significant[2] interactions reported in table 7[3]

Blend	Interaction of blend and storage temperature						Interaction of blend and storage period[4]	
	Log_{10} of fecal streptococci (organisms/g) at—			Log_{10} of mold count (organisms/g) at—			Log_{10} of thermophilic plate count (organisms/g) for—	
	25° C	37° C	49° C	25° C	37° C	49° C	Shorter	Longer
CSM (Public Law 480 blend).[5]	1.5ab	0.7a	0.7a	16a	0.8a	0.8a	1.6e	2.2d
CP(pre)WL	1.7a	.7a	.7a	1.3abc	.8a	.8a	2.6bc	2.2d
CP(full)WL	.7b	.7a	.7a	.8d	.7a	.8a	2.0de	2.2d
CCotW	1.0ab	.7a	.7a	1.0bcd	.8a	.7a	3.2a	3.4a
CCot(3)SW	.9ab	.7a	.7a	1.0bcd	.7a	.7a	3.1a	2.4cd
CCotSW	.8b	.7a	.7a	.9d	.8a	.7a	3.3a	2.8bc
CCot(⅓)SW	.7b	.7a	.7a	1.0bcd	.8a	.7a	2.2cd	2.6cd
CSW	.7b	.7a	.7a	1.0bcd	.9a	.7a	2.0de	2.5cd
CS (Public Law 480 blend).[5]	.8b	.7a	.7a	1.0bcd	.7a	.8a	1.9de	2.1d
CCot(soy)L	.8b	.7a	.7a	1.4ab	.8a	.8a	3.2a	2.6cd
CCot(cot)L	1.2ab	.7a	.7a	1.2bcd	.9a	.8a	3.0ab	3.3ab

[1]Each log_{10} is taken from a mean obtained by averaging the microbial count over the other variables. For example, the log_{10} of fecal streptococci for CSM at 25° C was obtained by averaging values over the different storage periods and antioxidant levels.

[2]Within a column, values followed by the same letter are not significantly different ($P < 0.05$) by Duncan's new multiple-range test.

[3]With storage period as the source of variation, results for interactions of blends and storage temperature were: Log_{10} of total plate count (organisms/g): 25° C, 3.4; 37° C, 2.5; and 49° C, 3.3 for shorter storage periods and 25° C, 3.7; 37° C, 3.5; and 49° C, 3.0 for longer. Log_{10} of thermophilic plate count (organisms/g): 25° C, 2.7; 37° C, 3.0; and 49° C, 2.1 for shorter storage periods and 25° C, 2.8; 37° C, 2.2; and 49° C, 2.6 for longer. Log_{10} of mold count (organisms/g): 25° C, 1.2; 37° C, 0.7; and 49° C, 0.8 for shorter storage periods and 25° C, 1.1; 37° C, 0.9; and 49° C, 0.7 for longer. These data could not be subjected to Duncan's new multiple-range test because the source of variation is quantitative.

[4]Shorter storage periods: 25° C, 6 months; 37° C, 3 months; and 49° C, 1 month. Longer storage periods: 25° C, 12 months, 37° C, 6 months; and 49° C, 2 months.

[5]The actual Public Law 480 blends contain added antioxidants.

the PAG guideline for total plate count, fecal coliforms, coagulase-positive staphylococci, and *Salmonella*. Other categories of organisms sometimes used to indicate food safety or quality—total coliforms, fecal streptococci, thermophiles, yeasts, molds, and *Clostridium perfringens* are also listed in table 10. No established standards are known to exist for these organisms in the type of food blends studied.

Changes in microbial levels during storage are reported in the analyses of variance results of table 7 and in the listed mean values of table 11 which refer to significant results of table 7. Storage temperature significantly influenced total plate count, total coliforms, fecal streptococci, thermophilic plate count, and mold (table 7). The means of all counts (not listed in table 11) except for thermophilic plate count declined continuously or stabilized at a given level as storage temperature increased. The means, averaged over the other experimental variables, indicate that thermophilic plate count increased from 1,725 at 25° C to 1,904 at 37° C and then declined to 781 at 49° C.

Analysis of variance also indicated that the effects of storage temperature were significant for total plate count, thermophilic plate count, and mold, dependent on storage time, and for fecal streptococci and mold, dependent on blend (table 7). But, in terms of numbers of organisms (which can be taking antilogarithms of the numbers listed in table 11, these differences have little importance to public health or in food spoilage. This is so even though in some instances we found in-

Table 12.—Blend-antioxidant level-storage treatment combinations compared by flavor panel[1]

Blend	Added antioxidants?	Combination used by storage treatment— Initial	25° C for 12 months	37° C for 6 months	49° C for 2 months
CSM (Public Law 480 blend.[2]	No	X	X	X	X
	Yes	X	X	X	X
CP(pre)WL	No	....	....	....	X
	Yes				
CP(full)WL	No	X	X	X	....
	Yes	X	X	X	X
CCotW	No	X	X	X	X
	Yes	X	....	X	X
CCot(3)SW	No	X	....	....	X
	Yes				
CCotSW	No	X	....	....	X
	Yes				
CCot(⅓)SW	No				
	Yes				
CSW	No	X	....	....	X
	Yes				
CS (Public Law 480 blend).[2]	No	X	....	....	X
	Yes				
CCot(soy)L	No	X	....	....	X
	Yes				
CCot(cot)L	No	X	X	X	X
	Yes	X	X	X	X

[1]X indicates combination compared by flavor panel.
[2]The actual Public Law blends contain added antioxidants.

creases in numbers of organisms with increasing storage interval or with increasing storage temperature.

Analysis of variance showed that storage time significantly affected only thermophilic plate count, dependent on blend (table 7). Although counts increased with increased storage time for only seven of the blends (table 11), the largest increase in mean count for the longer period over that for the shorter was less than 1,000 organisms/g. This difference could represent sampling variation in a particular blended food sample rather than a spoilage hazard. Analyses of variance did not reveal any significant effects of antioxidant level on any of the organisms investigated.

Because the dry blend samples were packaged in screwcap glass jars, no important changes in microbial levels during storage were anticipated. Mossel and Ingram (1955) have pointed out that an a_w as low as 0.70 makes microbial growth in foods unlikely. This is the a_w at 20° C when total moisture in legumes is 15%; in nonfat dry milk, 15%; in flour, 13% to 15%; and in starch, 18%. In our study, the moisture content of the blends averaged less than 10% throughout the storage time. A dry food is most likely to be spoiled by molds (Frazier and Westhoff 1978). But mold counts during our study showed little or no change during storage (table 11). The microbial stability of these blended foods, of course, depends on keeping them dry during storage.

Flavor evaluation

Comparisons of flavor scores were restricted to certain combinations of blend, temperature-time treatment, and antioxidant level (table 12). Analysis of variance results are presented in table 13, and means reflecting significant interactions of this table are listed in table 14. Care must be taken in interpreting the flavor-panel results because of nonorthogonal interactions; that is,

Table 13.—Analysis of variance of flavor panel scores for selected blends for varying antioxidant levels and storage treatment conditions

Sources of variation	Among samples varying in antioxidant level						Among samples varying in storage treatment											
	None added			Added			Initial			25° C for 12 months			37° C for 6 months			49° C for 2 months		
	Corn	Off-flavor	Overall	Corn	Off-flavor	Overall	Corn	Off-flavor	Overall	Corn	Off-flavor	Overall	Corn	Off-flavor	Overall	Corn	Off-flavor	Overall
Between different blends.	NS	**	NS	NS	NS	NS	NS	NS	NS	NS	*	NS	NS	NS	NS	NS	**	NS
Dependent on storage treatment.[2]	NS	*	NS	NS	*	NS	...	...	...	...	...	...	...	...	...	...	...	...
Dependent on whether antioxidants are added or not.	...	...	...	...	...	...	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Between different treatments.	NS	NS	NS	NS	**	*	...	...	...	...	...	...	...	...	...	...	...	...
Between no added antioxidants versus added antioxidants.	...	...	...	...	...	...	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

[1]Estimates of statistical difference are based on using, as the error term, the means square of the highest order interactions (*=$P<0.05$; **=$P<0.01$; NS=significance tested for but not found; all other interactions were inappropriate for tests of significance). All combinations of storage treatment and antioxidant level were tested. Of these, significance was found only for the storage treatment 49° C for 2 months and only for off-flavor with ($P<0.05$) and without ($P<0.01$) added antioxidants and for overall flavor quality with added antioxidants ($P<0.05$).

[2]Storage treatments were: initial, 25° C for 12 months, 37° C for 6 months, and 49° C for 2 months.

Corn—score for intensity of corn flavor.
Off-flavor—score for intensity of off-flavor.
Overall—score for overall flavor quality.

not all treatments are included in each blend average, and not all blends are included in each treatment average, etc. We did not test all possible combinations of blends, antioxidant levels, and storage treatments. For example, part of the blend effect may be caused by antioxidant level, since 27 samples had added antioxidants and only 15 samples did not. The most unbiased comparisons will be in those interactions where blend, storage treatment, and antioxidant level are specified. So, for example, comparison of blends without added antioxidants after storage at 49° C for 2 months is more unbiased than comparison of blends without added antioxidants, averaged over the same storage treatment (see table 14).

There were no significant differences among samples in scores for intensity of corn flavor (table 13). Significant differences occurred most often in scores for off-flavor. Analysis of variance results not reported in table 13 (which shows results for subsets of data) indicated a significant difference at $P<0.01$ between storage treatments, using all samples evaluated in the flavor study. Examination of means (not shown in table 14) indicated that the higher the temperature of storage treatment, the greater the off-flavor in most blends. CS seemed to be particularly stable against off-flavor development, as is seen in the mean score for storage at 40° C for 2 months and without added antioxidants. In this same comparison, the corn-cottonseed-lysine-monohydrochloride blends, especially the one containing soy oil, also had relatively low off-flavor scores. Apparently, use of a dairy product results in greater off-flavor development under more severe storage conditions (49° C for 2 months) when antioxidants are not added. Addition of antioxidants eliminated such differences.

Because of high peroxide development observed at 25° C in blends containing peanut flour (table 9), greater off-flavor development might be expected in those blends. And the off-flavor score for the sample of CP(full)WL with added antioxidants stored at 49° C for 2 months was notably high. But, in other comparisons involving peanut-containing blends, off-flavor scores were moderate or even low. Bookwalter et al (1979), in a study of peanut-fortified corn blends containing either one or two antioxidants and ranging in lipid content from 6.5% to 8.6%, found that blends containing peanut flour were generally a little higher in peroxide values than control blends containing soy flours. But, in that study, these higher peroxide values (even up to 37 meq/kg fat) were not associated with off-flavor during storage, as shown by flavor panel scores.

High free fatty acid values for cottonseed-containing blends (table 9) resulted in off-flavor scores that were markedly more intense than those for other blends.

Ratings for overall flavor quality decreased with rising storage temperature. In the limited comparison made among blends with added antioxidants after storage at 49° C for 2 months, CP(full)WL was given a significantly poorer score than were the three other blends (table 14).

CONCLUSIONS

This study illustrated the practical use of computer formulation for optimum protein quality by chemical score as one step in the development of nutritious blended foods containing glandless cottonseed or peanut flour. The approach permitted the rapid formulation of better quality protein blends for subsequent evaluation of protein quality by animal assay together with initial and storage screening with respect to color, flavor, texture, microbiological, and chemical-stability indices. From this screening, a formulation containing glandless cottonseed, CCot(cot)L, was judged the most promising of the series of Public Law 480 type corn-based blends using soy, soy-cottonseed, cottonseed, or peanut as the oilseed contributor. Peanut-containing blends could possibly be upgraded to a quality comparable to the best cottonseed-containing blends by reformulation with whey, better antioxidant protection, and several different essential amino acids at appropriate levels.

Table 14.—Flavor panel scores[1] reflecting significant[2] interactions reported in table 13[3]

Blend	Interactions of blend and antioxidant level (none added[4])—score for off-flavor	Interactions of blend and storage treatment at—		Interactions of blend, antioxidant level, and storage treatment at 49° C for 2 months when—		
		25° C for 12 months—score for off-flavor	49° C for 2 months—score for off-flavor	No antioxidants added—score for off-flavor	Antioxidants added—score for—	
					Off-flavor	Overall
CSM (Public Law 480 blend).[5]	4.8a	4.1a	3.7ab	6.6a	4.0b	3.7ab
CP(pre)WL	3.8abc	4.1ab	4.4bcd	....	....	....
CP(full)WL	3.2bc	2.5b	3.8ab	....	6.7a	2.9b
CCotW	3.6bc	3.5ab	5.1a	4.6abcd	4.0b	5.2a
CCot(3)SW	4.4ab	....	3.9ab	5.5abc	....	....
CCotSW	4.3ab	....	3.1b	4.9abc	....	....
CCot(⅓)SW						
CSW	4.2ab	....	3.8ab	6.1ab	....	....
CS(Public Law 480 blend).[5]	2.5c	....	3.7ab	2.3e	....	....
CCot(soy)L	3.3bc	....	3.7ab	2.8de	....	....
CCot(cot)L	4.1ab	4.2a	3.7ab	4.0cde	4.3b	3.9ab

[1]Each flavor-panel score is a mean obtained by averaging over the other variables. For example, the off-flavor value for CSM, when no antioxidants were added, was obtained by averaging values over different storage treatment conditions. Off-flavor—score for the intensity of off-flavor. The higher the score, the greater the intensity. Rating scale: 1, not detectable; 2, just detectable; 3, very slight; 4, slight-to-moderate; 5, moderate; 6, moderate-to-strong, 7, strong; 8, very strong; 9, extreme. Overall—score for overall flavor quality. The higher the score, the better the quality. Rating scale: 1, very poor, 2, poor; 3, slightly poor; 4, slightly fair; 5, fair; 6, fairly good; 7, good; 8, very good; 9, excellent.

[2]Within a column, means followed by the same letter are not significantly different ($P<0.05$) by Duncan's new multiple-range test.

[3]Where no data are given, the interactions were not evaluated by the flavor panel. With storage treatment as the source of variation, results for interactions of blend and added antioxidants were: off-flavor, 3.3 and overall, 5.0 for initial; off-flavor, 3.4 and overall, 4.6 at 25° C for 12 months; off-flavor, 4.2 and overall, 4.1 at 37° C for 6 months; and off-flavor, 4.9 and overall, 3.7 at 49° C for 2 months. When antioxidants were not added, differences were not significant.

[4]Interactions of blend and antioxidant level were not significantly different for either off-flavor or overall when antioxidants were added.

[5]The actual Public Law 480 blends contain added antioxidants.

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